QuantiGlo® ELISA

Human IL-2 Immunoassay

Catalog Number Q2000B

For the quantitative determination of human Interleukin 2 (IL-2) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Human Interleukin 2 (IL-2), also known as T cell growth factor (TCGF), is a 15-18 kDa variably glycosylated α-helical polypeptide that is a member of the common gamma chain (γc) cytokine family (1-4). It exists as a monomer and has a notably short half-life (< 30 minutes) (1). Human IL-2 is synthesized as a 153 amino acid (aa) precursor that contains a 20 aa signal sequence plus a 133 aa mature region (5, 6). The mature region is α-helical in nature, and contains one utilized O-linked glycosylation site at Thr3, plus three cysteines, two of which form an intrachain disulfide bond that is essential for activity (7). Mature human IL-2 shares 73%, 66%, 78%, and 97% aa identity with canine, rat, feline, and rhesus monkey IL-2, respectively. Although human IL-2 shares only approximately 60% aa identity with the highly polymorphic mouse IL-2, human IL-2 is known to be active on mouse IL-2 responsive cells. Cells reported to secrete IL-2 include γδT cells (8), activated conventional $CD4^+$ and $CD8^+$ T cells (1, 9), neurons (10, 11), microglia (12), and hematopoietic stem cells (13).

The receptor for IL-2 (IL-2 R) is composed of three subunits, the 55 kDa CD25/IL-2 Rα chain, the 70 kDa CD122/IL-2 Rβ chain, and the 65 kDa CD132/γc chain (1, 3). IL-2 first binds to CD25, the binary complex then recruits CD122 and CD132 to form the quaternary signaling complex (1, 14).

In vitro studies have shown an important role for IL-2 in T cell activation and expansion. *In vivo*, IL-2 is critical for the development, maintenance and function of regulatory T cells which provide protection against autoimmune disease. On the other hand, IL-2 can also promote autoimmune inflammation in target organs through its roles in regulating the expression of T cell trafficking genes, and production of Th2 cytokines. Within the $CD8^+$ T cell subset, IL-2 is essential for optimal primary responses and differentiation into terminal effector cells. IL-2 also promotes the development of activated $CD8^+$ T cells into memory cells. (1).

The QuantiGlo Human IL-2 Immunoassay is a 5.5 hour solid phase chemiluminescent ELISA designed to measure IL-2 levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-2 and antibodies raised against the recombinant factor. It has been shown to accurately quantitate recombinant human IL-2. Results obtained using natural human IL-2 showed linear curves that were parallel to the standard curves obtained using the recombinant QuantiGlo kit standards. These results indicate that this kit can be used to determine relative mass values for natural human IL-2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, an enhanced luminol/peroxide substrate solution is added to the wells and light is produced in proportion to the amount of IL-2 bound in the initial step. A microplate luminometer is used to measure the intensity of the light emitted.

TECHNICAL HINTS

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent and repeat the assay.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the QuantiGlo Immunoassay, the possibility of interference cannot be excluded.
- Variation in pipetting technique, washing technique, luminometers, incubation time or temperature can cause variations in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Relative light units (RLUs) may differ among luminometers. Adjust settings as recommended by the instrument manufacturer.
- Relative light units may vary within the 20 minute reading window.
- Due to limitations of many luminometers, it is recommended that the standards be assayed in duplicate from high to low beginning with the high standard in wells A_1 and A_2.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
IL-2 Microplate	893203	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against IL-2.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
IL-2 Conjugate	893204	21 mL of polyclonal antibody against IL-2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
IL-2 Standard	890681	12.5 ng of recombinant human IL-2 in a buffered protein base with preservatives; lyophilized.	
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a concentrated buffered protein base with preservatives.	
Wash Buffer Concentrate	895222	100 mL of a 10-fold concentrated solution of buffered surfactant.	
Glo Reagent A	895868	4 mL of stabilized enhanced luminol.	
Glo Reagent B	895869	8 mL of stabilized hydrogen peroxide.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Luminometer set with the following parameters: 1.0 minute lag time; 0.5 sec/well read time; summation mode; auto gain on, or equivalent.
- Pipettes and pipette tips.
- 100 mL and 1000 mL graduated cylinders.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards.
- Human IL-2 Controls (optional; available from R&D Systems).

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Hemolyzed samples are not suitable for use in this assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 100 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 1000 mL of Wash Buffer.

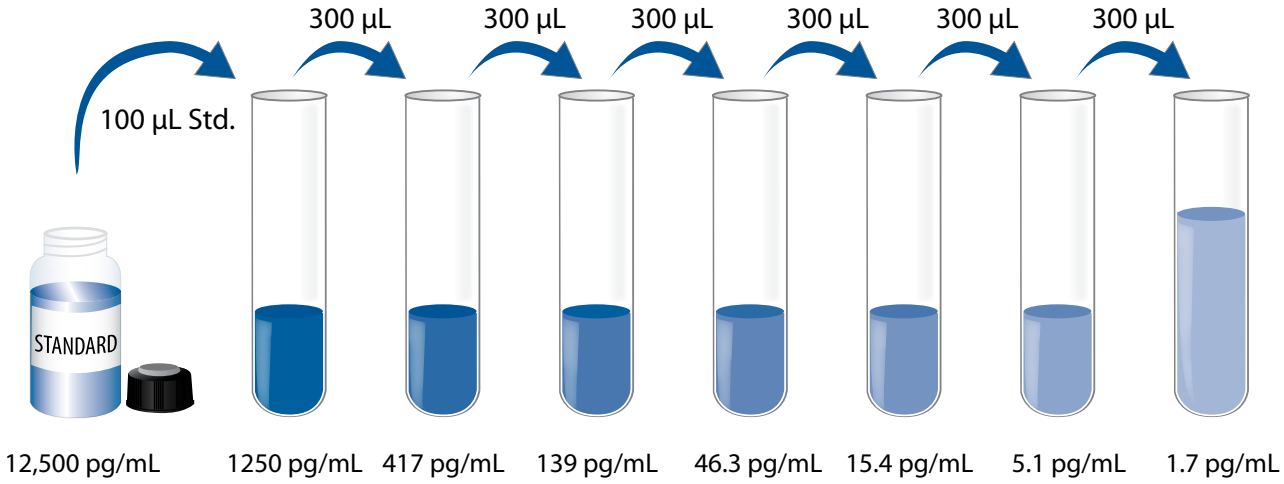
Working Glo Reagent - 1 part Glo Reagent A (4 mL) and 2 parts Glo Reagent B (8 mL) should be mixed together 15 minutes to 4 hours before use in a capped plastic container and protected from light. 100 µL of the resultant mixture is required per well.

Note: *If running the assay in less than 96 wells, mix appropriate amounts of Glo Reagent A and Glo Reagent B. To assay half a plate (48 wells), mix 2 mL of Glo Reagent A with 4 mL of Glo Reagent B. Working Glo Reagent should be discarded after use.*

Calibrator Diluent RD5P (1X) - Add 20 mL of Calibrator Diluent RD5P Concentrate to 80 mL of deionized or distilled water to yield 100 mL of Calibrator Diluent RD5P (1X).

IL-2 Standard - Reconstitute Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 12,500 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5P (1X) into the 1250 pg/mL tube. Pipette 600 µL of Calibrator Diluent RD5P (1X) into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 1250 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (1X) serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1W to each well.
4. Add 100 µL of Standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of IL-2 Conjugate to each well. Cover with a new adhesive strip. Incubate for 3 hours at room temperature on the shaker.

   Note: *Prepare Working Glo Reagent at this time.*
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Working Glo Reagent to each well. Incubate for 5-20 minutes at room temperature **on the benchtop. Protect from light.**
9. Determine the RLU of each well using a luminometer set with the following parameters: 1.0 min. lag time; 0.5 sec/well read time; summation mode; auto gain on, or equivelant.

CALCULATION OF RESULTS

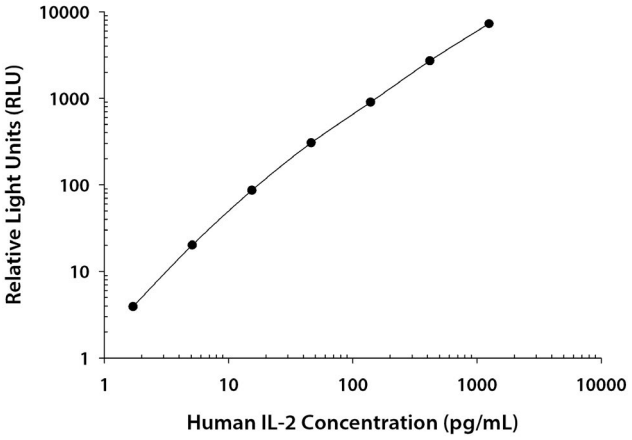
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard RLU.

Create a standard curve by reducing the data using computer software capable of generating a cubic-spline curve fit.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	RLU	Average	Corrected
0	1.13 1.21	1.17	—
1.7	4.48 5.69	5.08	3.91
5.1	20.3 22.3	21.3	20.1
15.4	86.0 89.0	87.5	86.3
46.3	302 312	307	306
139	889 915	902	901
417	2639 2773	2706	2705
1250	7153 7388	7271	7270

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	5.8	175	884	5.9	165	795
Standard deviation	0.19	5.6	23	0.46	11	59
CV (%)	3.3	3.2	2.6	7.8	6.7	7.4

RECOVERY

The recovery of recombinant human IL-2 spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	102	99-105%
Serum (n=4)	98	96-101%
EDTA plasma (n=4)	97	95-100%
Heparin plasma (n=4)	97	94-104%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of IL-2 in various matrices were diluted with the Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	101	99	100	100
	Range (%)	100-103	92-104	94-103	96-103
1:4	Average % of Expected	101	100	101	102
	Range (%)	98-105	94-107	95-104	97-105
1:8	Average % of Expected	98	102	104	103
	Range (%)	96-99	95-108	99-108	97-108
1:16	Average % of Expected	96	101	101	102
	Range (%)	92-103	92-108	101-102	95-107
1:32	Average % of Expected	95	101	101	101
	Range (%)	91-102	92-109	99-103	95-108

SENSITIVITY

Nineteen assays were evaluated and the minimum detectable dose (MDD) of IL-2 ranged from 0.03-0.25 pg/mL. The mean MDD was 0.1 pg/mL.

The MDD was determined by adding two standard deviations to the mean RLU of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL-2 produced at R&D Systems.

Both the NIBSC/WHO 1st International natural human IL-2 Standard 86/504, which was intended as a potency standard, and the interim reference recombinant human IL-2 standard 86/564 were evaluated in this kit. Both materials parallel the QuantiGlo Human IL-2 standard curve. To convert sample values obtained with the QuantiGlo Human IL-2 kit to relative approximate NIBSC units, use the appropriate equation below:

NIBSC (86/504) approximate value (IU/mL) = 0.013 x QuantiGlo Human IL-2 value (pg/mL)

NIBSC (86/564) approximate value (U/mL) = 0.010 x QuantiGlo Human IL-2 value (pg/mL)

Note: *Based on data generated in September 2008.*

SAMPLE VALUES

Serum/Plasma - Thirty-six samples from apparently healthy volunteers were evaluated for the presence of human IL-2 in this assay. No medical histories were available for the donors used in this study. All samples measured less than the lowest standard, 1.7 pg/mL.

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and stimulated with 10 µg/mL of PHA. Aliquots of the cell culture supernate were removed on days 1 and 6 and assayed for levels of natural human IL-2.

Condition	Day 1 (pg/mL)	Day 6 (pg/mL)
Stimulated	906	3.40
Unstimulated	ND	1.82

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human IL-2.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range IL-2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:
IL-2 Rα
IL-2 Rβ
IL-2 Rγ

Recombinant mouse:
IL-2
IL-2 Rγ

Other recombinants:
canine IL-2
cotton rat IL-2
feline IL-2
porcine IL-2
rat IL-2

Recombinant equine IL-2 cross-reacts approximately 0.1% in this assay.

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